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This grant provided partial support for a FASEB Summer Conference on "Microbial Pathogenesis: Mechanisms of Infectious Diseases" that was held July 4-9, 1998 at Snowmass, Colorado. This unique conference brought together more than 70 scientists who study the pathogenesis of infectious diseases caused by viruses, bacteria, fungi and parasites. The success of the conference is shown by the unanimous vote of all who attended to meeting to hold a similar conference after several years.

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Kathryn V Holmes 4/1/99

PI - Signature Date

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(5) INTRODUCTION

The FASEB Conference on "Microbial Pathogenesis: Mechanisms of Infectious Diseases" was held on July 4-9, 1998 at Snowmass, Colorado. This meeting was sponsored in part by award DAMD17-98-1-8652 from the US Army Medical Research and Materiel Command.

(6) BODY

This was the 3rd FASEB meeting on the pathogenesis of infectious diseases. The conference was managed by FASEB, which handled advertisements, registration, lodging, local arrangements, and all financial transactions. The scientific part of the conference was managed by the conference chairman, Dr. Kathryn V. Holmes, Professor, University of Colorado Health Sciences Center, Denver, Colorado and the vice-chair, Dr. William Goldman, Associate Professor, Washington University of St. Louis, St. Louis, Missouri.

Approximately 80 people attended the conference in Snowmass, Colorado. There were 32 plenary speeches and 21 posters at the meeting.

A unique feature of this conference is that it brings together scientists who study infections caused by all types of infectious organisms including viruses, bacteria, fungi and parasites. The principal topics addressed included: Attachment, colonization and penetration of microbes; the cell biology of infectious diseases, the genetics and regulation of virulence factors, host resistance to infection, host defenses, evolution and diversity of pathogens, and dynamic events in infected cells and tissues. The program of the meeting, which also includes abstracts of the posters, is attached here as an appendix.

Reports from the attendees to the chair showed that the meeting was scientifically valuable. There were lively and extended discussions that included many members of the audience. Many scientists who attended the meeting indicated that the conference had suggested significant changes in their research plans. There was unanimous agreement from the attendees that another conference on this broad topic should be held in two to three years.

(7) CONCLUSIONS

It is important to have periodic meetings to bring together scientists who study infectious diseases caused by different types of pathogenic microbes including viruses, bacteria, fungi and parasites. Many interesting common principles of infectious diseases and host responses were identified during the discussions at the meeting.

(8) REFERENCES

Not applicable

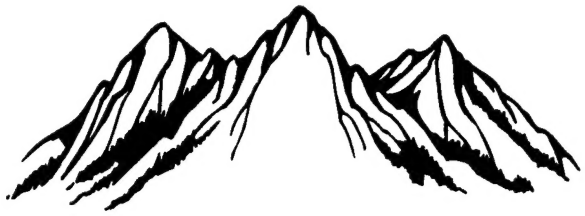
(9) APPENDICES

The Program of the meeting, which was distributed to all attendees, is included as an appendix to this final report.

BIBLIOGRAPHY AND LIST OF PERSONNEL

There were no publications generated by this meeting. The program and abstracts of the meeting were distributed to attendees and are included as an appendix to this report. Funds were distributed by FASEB to pay for transportation and accommodation of several plenary speakers at the meeting.

FASEB Summer Conference



Microbial Pathogenesis: Mechanisms of Infectious Diseases

Snowmass, Colorado
July 4-9, 1998

FASEB Summer Conference on
"Microbial Pathogenesis: Mechanisms of Infectious Diseases"

Sponsors

The organizers are grateful for the support for this conference that was generously provided by:

The Burroughs Wellcome Fund

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PROGRAM

Sunday morning, July 5

9:00 am — Kathryn Holmes, University of Colorado
Welcoming remarks

Attachment, Colonization and Penetration
Chair: Patricia Spear

9:30 — Patricia Spear *Northwestern University School of Medicine*
"Different co-receptors for entry of herpes simplex virus into different cell types"

10:10 — Rebekah Devinney *University of British Columbia*
"Exploitation of host cell pathways by enteropathogenic and enterohemorrhagic *E. coli*"

10:50 — Coffee break

11:10 — David Sibley *Washington University*
"Motility and invasion by Toxoplasma"

11:50 — Paula Sundstrom *Ohio State University*
"Covalent adhesion mediated by *Candida albicans* Hwp1, a substrate for mammalian transglutaminase"

12:10 — Lunch

Sunday evening, July 5

Cell Biology of Infectious Diseases
Chair: Jorge Galan

7:30 pm — Philippe Sansonetti *Institut Pasteur*
"Invasion of the epithelial barrier by Shigella: genes, effectors and signals"

8:10 pm — Norma Andrews *Yale University*
"Signal transduction and lysosome recruitment during host cell invasion by trypanosomes"

8: 50 pm — Jorge Galan *SUNY Stony Brook*
"Manipulation of the host-cell actin cytoskeleton by *Salmonella typhimurium*"

9: 30 pm — Amy Decatur *University of California*
"Possible Regulation of a bacterial virulence factor by a eukaryotic protein degradation signal"

9:50 pm — End of session

Monday morning, July 6

Genetic Determinants of Virulence

Chair: Virginia Miller

9:00 am	Virginia Miller	*Washington University*
	"Approaches to identifying virulence determinants- an overview"	
9:40 am	Mary Estes	*Baylor College of Medicine, Houston*
	"Viral enterotoxins and pathogenesis"	
10:20 am	Coffee break	
10:40 am	Brendan Cormack	*Stanford University*
	"The genetics of virulence in the pathogenic yeast *Candida glabrata*"	
11:20 am	Ron Taylor	*Dartmouth Medical School*
	"Genetic analysis of *Vibrio cholerae* colonization"	
12:00 am	Andrew J. Darwin	*Washington University*
	"Identification of *Yersinia enterocolitica* virulence genes in a murine model of infection by signature-tagged transposon mutagenesis"	
12:20	Lunch	

Monday evening, July 6

Regulation of Virulence

Chair: Jeff Miller

7:30 pm	Jeff Miller	*UCLA Medical School*
	"Signal transduction during the Bordetella infectious cycle"	
8:10 pm	Lee Shapiro	*University of Colorado Health Sciences Center*
	"Role of MAP kinases in HIV pathogenesis"	
8:50 pm	Russell Maurer	*Case Western Reserve University*
	"Regulation of salmonella invasion gene expression by the barA sensor-kinase and by RNA-mediated control of message degradation"	
9:10 pm	**Bernard N. Fields Memorial Lecture**	
	Bernard Moss	*National Institutes of Health*
	"Evasion of host responses to poxvirus infections"	
9:50 pm	End of session	

Tuesday morning, July 7

Resistance to Infection

Chair: Frank Chisari

9:00 am	Frank Chisari	*Scripps Research Institute*
	"Intracellular inactivation of the hepatitis B virus by inflammatory cytokines"	
9:40 am	Ferric Fang	*University of Colorado Health Sciences Center*
	"Interactions between *Salmonella typhimurium* and phagocyte oxygen-dependent antimicrobial systems"	
10:20 am	Coffee break	
10:40 am	JoAnne Flynn	*University of Pittsburgh School of Medicine*
	"Dendritic cells and macrophages in tuberculosis"	
11:00 am	Charles A. Scanga	*University of Pittsburgh School of Medicine*
	"Reactive mitrogen intermediates maintain latent *Mycobacterium tuberculosis* infection in mice"	
11:20 am	Jean-Paul Coutelier	*Christian de Duve Institute for Cellular Pathology*
	"Inhibition of T helper 2-dependent antibody responses during viral and parasitic infections"	
11:40 am	**Business meeting**	
12:10	Lunch	

5:00-6:00 pm	**POSTER SESSION**

Tuesday evening, July 7

Host Defenses

Chair: Michael Oldstone

7:30 pm	Michael Oldstone	*Scripps Research Institute*
	Overview: "Virus/microbe immune interactions"	
8:10 pm	Diane Griffin	*Johns Hopkins University School of Public Health*
	"Role of the immune response in determining the outcome of alphavirus infection of neurons"	
8:50 pm	Jack Griffin	*Johns Hopkins University School of Medicine*
	"Guillain-Barre Syndrome: What happens in the peripheral nervous system after Campylobacter infection"	
9:30 pm	Bryan Williams	*Cleveland Clinic Foundation*
	"The role of interferon regulated pathways in host resistance to disease "	
10:10 pm	End of session	

Wednesday morning, July 8

Evolution and Diversity of Pathogens

Chair: Margo Brinton

9:00 am	Robert Donald	*University of Pennsylvania*
	"Molecular genetic dissection of *Toxoplasma gondii* pathogenesis"	
9:40 am	Margo Brinton	*Georgia State University*
	"Co-infections of Simian Hemorrhagic Fever virus and Reston Ebola virus, an unfinished story"	
10:20 am	Coffee break	
10:40 am	Robert G. Webster	*St. Jude Children's Research Hospital*
	Influenza viruses	
11:20 am	Break for lunch and rafting trip	

Wednesday evening, July 8

Dynamic Events in Infected Cells and Tissues

Ashley Haas

7:30 pm	Ashley Haase	*University of Minnesota School of Medicine*
	"Viral and T cell dynamics in immune depletion in HIV-1 infection"	
8:10 pm	Stephen Leppla	*National Institutes of Health*
	"Interaction of anthrax toxin with cellular targets"	
8:50 pm	John Ireland	*Duke University Medical Center*
	"Host-Induced germination of pathogenic *B. anthracis* endospores"	
9:10 pm	End of session	

Thursday morning, July 9

New Agents of Infectious Diseases

Chair: Ian Lipkin

9:00 am	Ian Lipkin	*University of California, Irvine*
	"Pathogenesis of Borna virus"	
9:40 am	Tom Morrison	*University of Utah School of Medicine*
	"Role of CD14 in lipoprotein-mediated neutrophil activation"	
10:00 am	David Relman	*Stanford University*
	"Exploring microbial diversity in the context of human disease: what's out there?"	
10:40 am	Meeting ends	

ABSTRACTS

Identification of secreted proteins of *Mycobacterium tuberculosis* using a PhoA fusion approach

Irmgard Behlau and Andrew Wright
Tufts University School of Medicine
Boston, Massachusetts, USA

Secreted gene products play an important role in host-pathogen interactions. We have used a PhoA fusion approach to identify membrane-associated and secreted proteins produced by *M. tuberculosis*. We have constructed a *M. tuberculosis*-phoA expression library by cloning *M. tuberculosis* genomic DNA in three possible reading frames upstream of a leaderless E. coli phoA gene which cannot give rise to active alkaline phosphatase unless it is fused to sequences that allow its secretion. Plasmids containing *M. tuberculosis* inserts were transformed into a phoA deletion derivative of E. coli.

Using this approach, we have identified several secreted gene products with amino acid sequence homology to known signal sequences and one with DNA sequence homology to an open reading frame in a *Mycobacterium leprae* cosmid library. One fusion, D3-58:PhoA, specified a 48 kDa lipoprotein by 3H-palmitate labeling. By sequence analysis, it appears to be a unique, newly identified mycobacterial lipoprotein. We have retrieved the entire gene from a *M. tuberculosis* plasmid library. To characterize this protein, we have expressed the lipoprotein under the control of the pBAD promoter in E. coli. It encodes a 32-33 kDa protein. We plan to further characterize this lipoprotein.

This *M. tuberculosis*:PhoA expression library may prove to be a useful tool to selectively identify *M. tuberculosis* secreted proteins which may be involved in its transport mechanisms, pathogenicity, and antigenicity.

Identification of *Yersinia enterocolitica* virulence genes in a murine model of infection by signature-tagged transposon mutagenesis.

Andrew J. Darwin and Virginia L. Miller
Washington University
St. Louis, Missouri, USA

Yersinia enterocolitica is a human enteric pathogen with a tropism for lymphoid tissue. In a murine model of infection, systemic disease is the ultimate result of either an oral or intraperitoneal *Y. enterocolitica* infection. Several genes encoded on a well characterized virulence plasmid are known to be essential for this disease process. However, only a small number of chromosomal genes have been demonstrated to be important for virulence. In this study the signature-tagged mutagenesis technique was used to screen transposon mutants for survival defects in the murine model of infection. 2015 mutants, in pools of 96, were sceened for their ability to grow in the mouse spleen after i.p. infection. This was followed by a secondary screen of all possible attenuated mutants in four pools of 96, leading to the identification of 55 putative attenuated mutants. Phenotypic analysis indicated that only approximately half of these mutants have transposon insertions in the virulence plasmid. This was confirmed by DNA sequence analysis and demonstrates the ability of the technique to identify known virulence genes. The attenuated phenotype of the chromosomal insertion mutants was confirmed and quantified by individual assessment in the animal model. The partial DNA sequence of all of the inactivated genes was determined, leading to the identification of both previously known and unknown chromosomal virulence determinants. Furthermore, the results of this initial study suggest that signature-tagged transposon mutagenesis is a suitable technique to comprehensively screen the *Y. enterocolitica* chromosome for genes required for survival in an animal host.

SycN and YscB form a heteromultimer that operates as a chaperone for LcrE in *Yersinia pestis*.

J. B. Day and G. V. Plano
University of Miami School of Medicine
Miami, Florida, USA

Yersinia pestis is able to avoid the defenses of its host through the expression and delivery of a set of plasmid encoded anti-host proteins called Yops. In a tightly controlled process, Yops are exported from the bacteria via a type III secretion system and delivered directly into the cytoplasm of the target host cell. The secreted LcrE protein is required to block Yop secretion in the presence of calcium in vitro and prior to cell contact in vivo. In this study, we characterized the role of the *Y. pestis* sycN and yscB gene products in the regulation of Yop and LcrE secretion. Mutants specifically defective in the expression of SycN or YscB secreted Yops into the culture supernatant in the presence and absence of Ca2+; however, the amount of LcrE secreted was specifically reduced. The predicted amino acid sequence of both SycN and YscB showed significant similarity to the Yersinia SycE and SycH proteins and with PscB of *Psuedomonas aeruginosa*. Using co-immunoprecipitation techniques and the yeast two- and three-hybrid systems we demonstrated that SycN binds to YscB and that it is this binary complex that associates with LcrE. Deletion analysis indicated that amino acid residues 51 to 85 of LcrE were required for interaction with the SycN/YscB complex. Together, these results suggest that a complex composed of SycN and YscB functions as a specific chaperone for LcrE in Y. pestis.

Possible regulation of a bacterial virulence factor by a eukaryotic protein degradation signal

Amy Decatur and Daniel A. Portnoy
Department of Molecular and Cellular Biology and School of Public Health
University of California
Berkeley, California, USA

The pore-forming protein listeriolysin O (LLO) of the intracellular pathogen *Listeria monocytogenes* is an essential virulence determinant that allows the bacterium to escape from the host cell vacuole. Although both the vascuolar and plasma membranes contain the binding site for LLO (cholesterol), the plasma membrane remains undamaged during a wild type infection. In contrast, *L. monocytogenes* engineered to synthesize the related cytolysin perfringolysin O (PFO) from the extracellular pathogen *Clostridum perfringens* are cytotoxic to the host cell *in vitro* and avirulent *in vivo*. We report that LLO, but not PFO, contains a putative PEST-like sequence that we propose targets it for degradation within the host cell cytosol. Bacteria synthesizing a mutant LLO containing a 26 amino acid in-frame deletion that effectively removes the PEST-like sequence are capable of vacuolar escape, but, like bacteria synthesizing PFO, are cytotoxic to the host cell. This cytotoxicity phenotype cannot be explained by a difference in hemolytic activity between the wild type and mutant LLO proteins as measured by *in vitro* assays. Finally, in keeping with the transferability of PEST sequences, addition of the first 35 residues of mature LLO (which contain the PEST-like sequence) to PFO allows bacteria expressing this chimeric protein to grow intracellularly. Thus, *L. monocytogenes* may achieve the critical balance between efficient escape from a vacuole and avoidance of host cell damage of incorporating a eukaryotic protein degradation signal into a potentially toxic bacterial virulence factor.

Characterization of the exoU Locus From *Pseudomonas aeruginosa*.

V. Finck-Barbançon, T. L. Yahr, and D. W. Frank
Medical College of Wisconsin
Milwaukee, Wisconsin, USA

Pseudomonas aeruginosa is an opportunistic nosocomial pathogen and the most common gram-negative causative agent of late-onset ventilator-associated pneumonia. This pathogen is also the leading cause of death in patients with cystic fibrosis, low or suppressed immunological defenses such as severe burns, patients with neoplastic diseases or critically ill. Recent experiments have shown that P. aeruginosa strains that are highly cytotoxic in vitro damage the lung epithelium in vivo. The specific virulence determinant responsible for epithelial damage in vivo and cytotoxicity in vitro has been mapped to the exoU locus. The present studies were focused on a genetic characterization of the exoU locus. Complementation of an ExoU mutant and Northern blot analysis suggested that the exoU locus may be organized as an operon. We extended the DNA sequence analysis downstream of exoU to determine if other open reading frames (ORFs) contributed to the expression of ExoU. Several downstream ORFs were identified after the translational stop codon of exoU. T7 expression analysis of His-tagged constructs encompassing either exoU alone or exoU and the downstream ORFs, showed that the larger construct resulted in concomitant expression of ExoU and a small associated protein of 15 kDa. This associated protein was isolated and subjected to amino terminal amino acid sequence analysis which identified the open reading frame expressed. This open reading frame encodes a protein of 137 amino acids whose translational start overlaps the stop codon of exoU. The presence of the conserved C-terminal motif found in the Syc and Syc-like family of chaperones in this small acidic (pI 4.4) protein, together with our complementation data and the T7 expression analysis, suggests that the 15 kDa protein is the chaperone for ExoU, SpcU (Specific Pseudomonas chaperone for ExoU).

Cell density sensing in *Histoplasma capsulatum*: A means to control virulence?

Linda Groppe Eissenberg, and William E. Goldman,
Department of Molecular Microbiology
Washington University School of Medicine
St. Louis, Missouri, USA

Histoplasma capsulatum spontaneously generates avirulent variants during broth culture as well as after entry into epithelial cells. The avirulent variant survives inside epithelial cells, establishes persistent infections of macrophages in cell culture, and may be responsible for persistent infections of humans and other mammals. While some signal unique to the environment within epithelial cells theoretically triggers generation of the variants, we wondered why they are also generated in liquid culture. We now have evidence that this pathogenic fungus regulates its virulence-associated phenotypes by sensing its cell density in broth, and we speculate that the same mechanism may also be used inside epithelial cells.

After 3 passages of virulent *H. capsulatum* at low density (5x 10^6 yeasts/ml), 80% of the organisms had converted to an avirulent phenotype (smooth colonies, dispersed growth in broth, absence of cell wall α-[1,3]-glucan). In contrast ≤0.5% of yeasts passaged 3 times at medium density (2.5 x 10^7/ml) or high density (1.25 x 10^8/ml) acquired an avirulent phenotype. In fact, within 24 hours (3 generations) of transfer to low density, 70-90% of yeasts lacked cell wall α-(1,3)-glucan as determined by immunoflourescence. We then quantitated the relative amount of α-(1,3)-glucan/yeast by spectrofluorimetry using flourescein-tagged antibodies to monitor α-(1,3)-glucan content and Texas Red-tagged antibodies to estimate the total number of yeasts. After incubation at low density, an initial precipitous drop in α-(1,3)-glucan content was followed by an increase only when a critical density of organisms (~2x10^8 yeasts/ml) was reached in late log phase. The phenotypic switch induced by culturing at low density was prevented by addition of filtrates from high density cultures.

Similar phenotypic modulation may occur when virulent *Histoplasma* infect epithelial cells: yeasts may sense their cell density is low, thus triggering a switch to the avirulent phenotype. This proposal is consistent with the observation that only avirulent yeasts are harvested from epithelial cells which are initially infected with a virulent inoculum. We speculate that a variety of intracellular pathogens may use "quorum sensing" mechanisms to detect that they are inside of host cells and correspondingly adjust their phenotype to one more suited for the new environment.

IKappaB Kinase Complex is an Intracellular Target for Endotoxic Lipopolysaccharide (LPS) in Human Monocytic Cells

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Nashville, Tennessee

Nuclear Factor NF-kappaB (NFkB/Rel) transription factors sequestered in cytoplasm by their inhibitors (IkB) mediate signaling to the nucleus in response to inflammatory, immune, and oxidant stimuli. We provided initial evidence that LPS induces phosphorylation of IkB alpha in human monocytic cells followed by its proteolysis via ATP-dependent proteosomal pathway (JBC 1993, 1995). Now, we present the evidence that IkB Kinase complex is activated by LPS in human monocytic THP-1 cells. The in situ kinase assay developed by us is specific for phosphorylation of the inhibitor at Ser-32 and Ser-36.

The activation of IkB kinase by LPS was concentration and time-dependent and was paralleled by degradation of IkB alpha and by nuclear import of NF-kB/Rel complexes. We have demonstrated using polyclonal antipeptide antibodies against recently discovered IKK alpha and IKK beta that these two kinases are part of IkB kinase complex activated by LPS. Thus, IkB kinase complex constitutes the main intracellular target for LPS-induced signaling to the nucleus.

Host-induced germination of pathogenic *B. anthracis* endospores

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Durham, North Carolina, USA

Anthrax is a rapidly progressing disease with fatalities often reported to occur within days. The initial step of an anthrax infection is the introduction of metabolically-dormant endospores into the body. *B. anthracis* endospores are assumed to break dormancy, a process termed germination, when they encounter specific environmental signals. Germination precedes vegetative outgrowth, and the corresponding expression of virulence genes (e.g., the anthrax toxin complex and capsule) responsible for subsequent pathologies. In general, the germination-responses of the Bacilli varies between species and can utilize a wide variety of signal factors/conditions. We show evidence that *B. anthracis* endospores possess a unique germination-response pathway that is triggered, within minutes, by exposure to cultured macrophages. The molecular signals triggering *B. anthracis* endospore germination are composed of components both contributed by sera and the host cells themselves. Non-pathogenic control *B. subtilis* endospores do not germinate under these conditions. Transposon-mutants of *B. anthracis*, specifically deficient in the host-induced germination-response, were isolated that are unable to germinate in macrophages while retaining identical germination and growth rates in bacterial media, as compared to the isogenic parent strains. We conclude that the pathogen, *B. anthracis* has evolved a germination-response system that recognizes specific factors present in the host microenvironment, and that this rapid germination event may be central to the establishment of infectious loci.

Inhibition of T helper 2-dependent antibody responses during viral and parasitic infections

D. Markine-Goriaynoff[1], T. D. Nguyen[2], P. Monteyne[1], M. El Azami El Idrissi[1], G. Bigaignon[2], J. P. Coutelier, [1]
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[2] Microbiology Unit, Cliniques Universitaires Saint-Luc, Université Catholique de Louvain, Bruxelles, Belgium

The development of immune disorders such as autoimmune diseases and allergies may depend on the balance between T helper lymphocyte (Th)1 and Th2 responses elicited in the affected organism. In the mouse, several distinct viral infections lead to the modulation of simultaneous non antiviral immune responses, resulting in a selective suppression of Th2-dependent antibody responses, including autoantibodies, in favor of the Th1 pathway. A similar effect was observed in animals infected with the parasite *Toxoplasma gondii.* That this phenomenon corresponds to an alteration of Th differentiation was shown by the observations that viruses affected only T-dependent antibody responses and that infection resulted in an inhibition of the expression of Th2 cytokines such as interleukin-4 and interleukin-9 in response to immunization with a non-viral protein antigen. To determine whether the infectious process by itself was required in order to modulate Th differentiation, antiviral antibody responses elicited by immunization with inactivated lactate dehydrogenase-elevating virus (LDV) and infection with live virus were compared. The results indicated that inactivated virus induced an antibody response with an isotypic distribution similar to that of anti-protein responses. In addition, live virus could depress the IgG1 antiviral antibody production following inoculation of inactivated virus, indicating that infection was required for the inhibition of Th2-dependent responses. Finally, the interleukin-4 and interleukin-9 inhibition observed after infection with LDV was not induced by this virus in mice deficient for the gamma-interferon receptor gene. Thus, the modulation of Th responses triggered by such infectious agents is a consequence of gamma-interferon secretion resulting from infection. These results indicate that infections with viruses or parasites may affect concomitant unrelated immune responses and are therefore susceptible to modify the outcome of immune-mediated diseases.

Regulation of salmonella invasion gene expression by the barA sensor-kinase and by RNA-mediated control of message degradation

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+Dept. of Microbiology, Pathology, and Parasitology, College of Veterinary Medicine
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Environmental and genetic control of the expression of *Salmonella typhimurium* invasion genes is thought to be integrated at the level of hilA, a positive control factor for expression of these genes. Here, we report that barA, encoding a member of the two-component sensor kinase family, is also required for full expression of invasion genes. In a barA null mutant, expression of lacZ reporter fusions to several invasion genes is reduced three- to four-fold at pH6 and five- to twenty-fold at pH8. Invasion of cultured HEP2 cells is also reduced in the mutant. These phenotypes of the barA mutant may be attributed in part to inadequate expression of hilA. A lacZ fusion to hilA is reduced three fold at pH6, and invasion capacity of the mutant is restored by constitutive expression of hilA. However, at pH8 there is little (<2-fold) effect of the barA mutation on hilA expression, suggesting that under this condition, barA can modulate invasion gene expression other than by affecting transcription of hilA. Overproduction of sirA, another known positive regulator of hilA, suppresses the gene expression of defects of the cascade leading to invasion gene expression. We suggest that the function of barA, but not that of sirA, is partially redundant in Salmonella because the invasion gene expression defects of a sirA mutant are more severe than those of the barA mutant, at least under some conditions.

Another factor that affects invasion gene expression in Salmonella is the homologue of the csrA-csrB system that regulates glycogen synthesis in E. coli. The csrB gene in Salmonella is located in the vicinity of barA, but is oriented convergently with respect to barA and is separated from it by about 3 kb, so its expression is likely to be independent of barA. In E. coli, csrA encodes a protein factor that targets selected mRNAs for degradation, whereas csrB encodes a structural RNA that sequesters CsrA and thus stabilizes the same messages. In Salmonella, a csrB mutation reduces invasion gene expression, as does overproduction of E. coli CsrA. These findings suggest that the csr system works similarly in Salmonella as in E. coli. Overproduction of csrB in Salmonella strongly stimulates invasion gene expression from barA and sirA mutants, suggesting csrA-csrB act below sirA, possibly at the level of hilA or directly on invasion gene messages. CsrA-csrB gives Salmonella the ability to amplify the transcriptional regulation of invasion genes by a further choice of degrading or stabilizing the target messages.

Role of CD14 in lipoprotein-mediated neutrophil activation

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Borrelia burgdorferi possess membrane lipoproteins that exhibit stimulatory properties and consequently have been implicated in the pathology related to Lyme disease. During infection with *B. burgdorferi*, neutrophils are the predominant infiltrate into the inflamed arthritic joint, and may play a role in both clearance and pathogenesis. In vitro, lipoproteins can directly prime and activate neutrophils, and therefore lipoprotein-neutrophil interactions could play a critical role in host response to *B. burgdorferi* infection (Morrison, Weis et al. 1997). As CD14 mediates signaling by a number of lipid-modified bacterial products, the involvement of CD14 in neutrophil activation by a *B. burgdorferi* lipoprotein, OspA, was determined. CD14-specific antibodies that blocked LPS-mediated signaling also inhibited OspA-dependent signaling, but not FMLP signaling. Isotype control antibodies and CD14-specific antibodies that do not block LPS-mediated signaling had no detectable effect on lipoprotein-mediated signaling. Antibody treatments which prevented 100 ng/ml LPS activation of neutrophils (>100-fold above normal), inhibited the OspA dose-response an average of 5-fold. These results indicate that lipoprotein activation of neutrophils was not as dependent on CD14 as was LPS signaling.

In vivo structure function analysis of the _Pseudomonas aeruginosa_ ADP-ribosyltransferase, exoenzyme S.

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Exoenzyme S (ExoS, 453 amino acids) catalyzes FAS-dependent ADP-ribosylation of several eukaryotic proteins, including both extracellular and intracellular proteins. Molecular and biochemical studies have shown that ExoS is organized into two domains. The amino terminus (amino acids 1-220) possesses limited homology with the YopE cytotoxin of *Yersinia pseudotuberculosis*, while the carboxyl terminus (amino acids 232-453) possesses the FAS-dependent ADP-ribosyltransferase domain. Recent studies have reported that expression of ExoS is cytotoxic to eukaryotic cells. To gain insight into the role of ADP-ribosylation and YopE-like effects in the cytotoxicity of ExoS, the effect of expression of the ADP-ribosyltransferase domain and the YopE homologous domain of ExoS in Chinese Hamster Ovary (CHO) cells were determined.

The FAS-dependent ADP-ribosyltransferase domain ExoS (DN222), a catalytic mutant of DN222 (DN222-E381A) that is 2000 fold less active in ADP-ribosyltransferase activity, and the YopE homologous domain of ExoS (DC234), were transiently expressed in CHO cells under the regulation of the CMV promoter (pN222-E381A, and pC324). Lysates of CHO cells transfected for 5 hours with pN222 demonstrated a significantly increased level of ADP-ribosyltransferase activity over background. A time course of pN222 transfected CHO cells showed that ADP-ribosyltransferase activity increased over the first 6 hours of transfection, but decreased by 24 hours post-transfection. Cotransfection of pN222 with reporter plasmids, encoding the Green Fluorescent Protein (GFP) or Luciferase, demonstrated that expression of the ADP-ribosyltransferase domain of ExoS resulted in a dose-dependent reduction of both the steady state levels of GFP and the number of transfectants expressing detectable levels of GFP, and Luciferase expression. Cotransfection of the catalytic mutant, pN222-E381A or a control plasmid had no effect on reporter protein levels. Trypan blue exclusion analysis of transfected cells indicated that intracellular expression of DN222, but not DN222-E381A, was lethal. Similar to YopE, intracellular expression of DC234 resulted in a rounded morphology of the CHO cells, but did not inhibit reporter protein expression. This altered morphology was not seen with expression of DN222 or DN222-E381A. Taken together, these data suggest that the ADP-ribosyltransferase activity of ExoS is cytotoxic to eukaryotic cells and that two domains of Exoenzyme S cause distinct, separate effects on eukaryotic cells.

Protective activity of Clostridium antigenic preparations

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Immunosera were obtained from the following antigenic preparations from 18 h-old cultures of *Clostridium difficile* drown at 37°C in anaerobic conditions. Following preparations were made; cellular extract (CE), formaldehyde-treated cell suspension with 0.5% of formol (FC); and heated cells boiled for 1 h (OC). The immunosera were collected following injection of white rabbits with the antigen preparations. Agglutination titters were CE: 1:16, FC 1:476 and OC: 1:64. Passive immunization was carried out by injecting by the i.p. route groups of mice with 0.5 mL of the sera followed by challenging them 6 and 24 h later with a spore suspension. Both immunized and nonimmunized animals were observed for 4 days. Anti-CE and anti-FC protected 100% of the animals, while anti-OC protected 50% of mice. No difference was observed in mice challenged 6 or 24 h later.

The results indicated that the CE and FC, but not OC, induces a dose-dependent IL-6 production in murine spleen cells. Furthermore, splenic cells incubated with various concentrations of CE or FC did not elicit gamma interferon or tumor necrosis factor alpha or beta. In parallel, toxin A isolated form *C. difficile* was examined and comparable data with that of CE and FC was obtained. The data may, therefore, indicate that the toxin A is a major component of the CE and FC preparations of *C. difficile* in cytokine production.

Intestinal IL-15 expression in experimental human cryptosporidosis

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Background: Cryptosporidosis is one of the leading causes of chronic diarrhea worldwide. In normal immunocompetent hosts, it presents as a self-limited infection, but in immunocompromised hosts (e.g., AIDS) cryptosporidosis causes chronic diarrhea, wasting, and death. IL-15 I a cytokine which shares receptor subunits (β and γ) and functional activity with IL-2. IL-15 is reported to be produced by intestinal epithelial cell lines in vitro, however, its role in human enteric infections is unknown.
Methods: To assess the role of IL-15 in the intestinal immune response, jejunal biopsy specimens were obtained from healthy adult volunteers experimentally infected with *Cryptosporidium parvum*. Biopsies were also obtained from 2 AIDS patients with naturally-acquired chronic cryptosporidosis. Intestinal biopsies were examined by in situ hybridization using ^{35}S-labelled riboprobes. Available data include cryptosporidal antibody status prechallenge (AB), oocyst excretion and clinical symptoms.
Results: IL-15 message was only detected on biopsies obtained 3-16 days post-challenge. IL-15 mRNA was detected in discrete cells at the base of the epithelium (possibly antigen presenting cells). All 8 prechallenge biopsies were negative as were 6 biopsies obtained more than day 16 post-challenge. None of the biopsies from 7 AB+ volunteers had measurable IL-15 message. Among symptomatic AB- volunteers, 4/8 biopsies from days 3-16 post-challenge were positive. Neither of the 2 biopsies from asymptomatic volunteers nor the 2 from AIDS patients were positive.
Conclusions: The fact that IL-15 was only found in AB- volunteers suggests that IL-15 may play a role in priming the immune response in *cryptosporidium* naive individuals undergoing initial infection.

Reactive nitrogen intermediates maintain latent *Mycobacterium tuberculosis* infection in mice

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Mycobacterium tuberculosis (Mtb) is a facultative intracellular bacterium which replicates in macrophages and is the causative agent of tuberculosis. Most Mtb infections are controlled by the host and result in a latent infection which is characterized by the persistence of a few viable but presumably dormant mycobacteria. Although most latent infections never reactivate, a significant portion does and results in reactivation tuberculosis which is associated with high rates of morbidity, mortality and infectivity. Immunosuppression (e.g. aging, treatment with steroids, or HIV coinfection) is a risk factor for reactivation.

Little is known about the precise immunologic response necessary to maintain a latent Mtb infection. In mice, macrophage-derived reactive nitrogen intermediates (RNI) generated by inducible nitric oxide synthase (iNOS) have been shown to be required for resistance to acute Mtb infection. We have used two murine models of latent Mtb infection to investigate the role of RNI in latency. In the "low-dose" latency model, C57Bl/6 mice are infected with relatively low inoculum and stable numbers of mycobacteria are maintained for many months. Aminoguanidine (AG), a competitive inhibitor of iNOS, exacerbated this stable infection and the mice succumbed to tuberculosis with a mean survival time (MST) of 62 days. In the "drug-induced" latency model, also referred to as the Cornell model, C57Bl/6 mice are infected with Mtb and after three weeks, are treated with the antimycobacterial drugs isoniazid and pyrazinamide resulting in undetectable numbers of bacilli. In this model, AG administered after antibiotic therapy caused a 500 to 1000-fold increase in bacterial numbers in the lungs. However, mice did not succumb to the reactivating infection, even after 180 days of AG treatment. Most interestingly, the number of bacilli appeared to plateau in the lungs after 80 days. This effect is being confirmed using a different iNOS inhibitor, N6-(1 -iminoethyl)-L-lysine (NIL). RT-PCR analysis of RNA from lungs and spleens demonstrated expression of interferon-g, TNF-α and iNOS both during latency and throughout reactivation. These results suggest that an iNOS-independent mechanism may evolve during reactivation in the "drug-induced" model which is capable of controlling a reactivating infection. This finding may be of special relevance in human tuberculosis for which the role of iNOS is controversial.

I have used the broadly immunosuppressive glucocorticoid dexamethasone (DEX) to characterize the pattern of reactivation in the "drug-induced" model. Surprisingly, 100 days of DEX treatment (0.08 mg/mouse/day) induced only slight reactivation. In another experiment, hydrocortisone acetate (1.5 mg/mouse, s.q., q3d for 12.5 weeks then 0.5 mg/mouse q1d for 5.5 weeks) also failed to induce substantial reactivation. Thus, broad immunosuppression has little effect on latent Mtb infection in the "drug-induced" model while more precise inhibition of iNOS results in dramatic reactivation. These data underscore the critical role played by RNI in maintaining Mtb latency in mice.

Defining the role of a calcium-binding protein (CBP) secreted by *Histoplasma capsulatum*

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Histoplasma capsulatum is a dimorphic fungus which exists as a saprophytic mold in soil, producing condia and hyphal fragments which are easily aerosolized and inhaled. Once inside the lung, the fungus converts to a parasitic yeast phase which is capable of growing within alveolar macrophages. The mechanisms by which *Histoplasma* survives and proliferates in phagolysosomes are unknown but may be attributed, in part, to the production of a yeast-phase specific extracellular protein known as calcium-binding protein (CBP). We hypothesize that this abundant protein is important for Ca^{2+} acquisition by yeasts inside the phagolysosomal compartment since (1) the yeast form, but not the mycelial form is capable of growing in a calcium-limiting environment; (2) purified CBP facilitates the uptake of $^{45}CaCl_2$ by yeasts; and (3) studies with other pathogens suggest that calcium concentrations in phagolysosomes are low.

A set of genetic tools including telomeric shuttle vectors have been developed for *Histoplasma* by our laboratory. Transformation of *H. capsulatum* with a linearized telomeric plasmid results in extrachromosomal maintenance of the plasmid at high, copy number. We constructed a telomeric plasmid containing a selectable *URA5* gene and several kilobases of genomic DNA including the *CBP1* gene with an internal fragment replaced by a hygromycin resistance marker (*hph*). This construct was used to transform a uracil auxotroph of a virulent strain of *H. capsulatum*.. 5-fluoro-orotic acid was used to select for chromosomal integration of *CBP1::hph*. Allelic replacement of *CBP1* was confirmed through Southern blot analysis and PCR. Culture supernatants from this *H. capsulatum CBP1* null mutant show no evidence of CBP in a $^{45}CaCl_2$ binding assay. Also, this CBP mutant was not capable of growing in media deprived of calcium by EGTA. These experiments are the first molecular genetic test of a *Histoplasma* protein's function, and the initial results suggest a role for CBP in virulence. Further studies to determine the fate of this mutant in macrophages will shed more light on the role of CBP in pathogenesis.

Genetic heterogeneity of *Borrelia burgdorferi* clinical isolates: Correlation to clinical disease

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Whole genome RFLP and plasmid analyses were carried out on 36 *B. burgdorferi* isolates from the blood or skin lesions of patients with early Lyme disease. The majority of isolates (64%) had identical genetic profiles with the remaining isolates falling into four other genetic groups. A unique characteristic of the major group was the lack of a 38 Kb linear plasmid. The likelihood of patients with disseminated Lyme disease being infected with an isolate lacking the 38 Kb plasmid was very high ($p<.01$). The data suggest that absence of the 38 Kb linear plasmid correlates with a more invasive subtype of *B. burgdorferi.* Current studies are focused on identifying differences in gene expression patterns between the different genotypic variants of *B. burgdorferi* with the goal of uncovering potential pathogenic determinants.

The use of Dictyostelium to study host requirements for growth of the intracellular bacterial pathogen *Legionella pneumophila*

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L. pneumophila is a bacterial pathogen that causes a severe pneumonia in human beings. *L. pneumophila* is digested by phagocytic cells, but the bacteria evades the normal phagocytic pathway and instead replicates within the phagosome. The molecular basis for this ability is unknown. *L. pneumophila* can grow within a wide variety of phagocytic cells, including human and mouse macrophages, and its natural hosts, fresh water amoeba.

We have discovered how to grow *L. pneumophila* on the single celled slime mold, *Dictyostelium* discoideum. *L. pneumophila* growth in dictyostelium resembles its growth within macrophages and amoeba. The bacterial titer increases two to three logs in the course of 4-6 days. Electron microscopy demonstrates that the bacteria are replicating within a membrane-bound compartment. *L. pneumophila* growth in *Dictyostelium* also requires dot genes, which are *L. pneumophila* genes required for growth in macrophages.

In *Dictyostelium* it is possible to make mutations and determine the loci in which those mutations occur. We are beginning to screen for *Dictyostelium* mutants that no longer support the growth of *L. pneumophila*. This should point us to host functions that are involved in the growth of this intracellular pathogen.

Covalent adhesion mediated by *Candida albicans* Hwp1, a substrate for mammalian transglutaminase

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The indigenous commensal *Candida albicans* is emerging as a major human opportunistic pathogen because of increasing numbers of immunosuppressed individuals. Progression from commenalism to candidiasis involves invasion of keratinized epithelial cells by hyphae. Loss of innate and specific immune host defenses is primarily responsible for susceptibility to opportunistic candidiasis arising from endogenous *C. albicans* in the normal flora. However, pro-adhesive and pro-invasive factors of *C. albicans* also contribute to disease by mediating proliferation and penetration of host tissues when risk factors are present.

In efforts to understand the role of surface proteins in tissue invasion by *C. albicans*, we cloned HWP1, a unique surface protein gene expressed in germ tubes and true hyphae. HWP1 encodes an outer mannoprotein, Hwp1, with a cell surface-exposed, ligand-binding domain at the N-terminus and C-terminal features that confer covalent integration into the b-glucan of the cell wall. The composition of the N-terminal amino acid repeats is reminiscent of mammalian transglutaminase substrates and Hwp1 serves as a substrate in cross-linking reactions mediated by mammalian transglutaminase.

Transglutaminases in general catalyze intermolecular crosslinks by the formation of highly stable isodipeptide bonds between the g-carbonyl group of glutamine and the e-amino group of lysine residues, which are resistant to proteases, SDS and heat. Epithelial cell transglutaminases are important for the formation of cornified envelopes of mature squamous epithelial cells. To determine if Hwp1 is involved in transglutaminase-mediated cross-linking of *C. albicans* to BEC's homozygous hwp1/hwp1, and heterozygous HWP1/hwp1 mutant strains were compared to HWP1/HWP1 wild-type strains in stabilized adhesion assays that involve dissociation of loosely attached fungi from BEC's, by treatment with heat in the present of SDS. Stabilized adhesion of the homozygous hwp1/hwp1 mutant strain was reduced by 75% compared to strains expressing at least one intact HWP1 gene. Stabilized adhesion of *C. albicans* to BEC's through cross-links between Hwp1 and unidentified proteins on BEC's is a novel interaction between microbes and mammalian cells that has not been previously described.

The importance of HWP1 in candidiasis was further supported by an increase in survival in mice intravenously injected with the homozygous hwp1/hwp1 mutant compared to the other strains. An intriguing aspect of these findings is the possibility that invasion associated with systemic candidiasis is enhanced through interactions of surface Hwp1 with a plasma transglutaminase factor XIII, clot stabilizing factor or other host transglutaminases. These findings also suggest that inhibition of expression of Hwp1 on hyphal surfaces or interference with transglutaminase-mediated cross-linking could provide new strategies for therapeutic intervention in candidiasis.

Does *Salmonella typhimurium* produce murine typhoid and bovine enteritis using identical virulence mechanisms?

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S. typhimurium is able to infect a wide variety of warm blooded hosts but causes different signs of disease in different animal species. Calves infected with *S. typhimurium* develop severe diarrhea, with fatalities occurring from the resulting dehydration and intestinal lesions. In contrast, *S. typhimurium* does not cause diarrhea in mice but produces a systemic infection, designated murine typhoid, which is characterized by rapid bacterial growth in the reticuloendothelial system. The different signs of disease observed in mice and calves raise the question whether *S. typhimurium* uses different virulence mechanisms to produce illness in these hosts. To address this point we screened a bank of 260 *S. typhimurium* signature tagged transposon mutants, a random sample representing about 6% of the genome, for virulence in both mice and calves. In our screen 26 mutants were not recovered from organs of either mice or calves. However only 10 mutants were missing from organs of both hosts. The remaining 16 mutants were missing only from mice or only from calves. The genetic analysis of these attenuated mutants identified which virulence mechanisms are important for *S. typhimurium* infection of mice, calves or both hosts. This information is relevant for both the development of live attenuated Salmonella vaccine candidates for cattle and for understanding the basis of host adaptation.

How does an old virus learn new tricks? Dave Wentworth, Jeanne H. Schickli, Bruce Zelus, Kathryn V. Holmes, Dept. of Microbiology, University of Colorado Health Sciences Center

Murine biliary glycoprotein MHVR (Bgp1a) serves as the receptor for murine coronavirus MHV-A59 during acute infection of murine 17 Cl 1 cells. FACS analysis with monoclonal anti-receptor antibody MAb-CC1 showed that murine 17 Cl 1 cells persistently infected with MHV-A59 express very low levels of MHVR in comparison with uninfected 17 Cl 1 cells (Sawicki, *et al.*, 1995). Unlike MHV-A59, the virus from the persistently infected cells (MHV/BHK) infected cell lines derived from hamsters, humans, cats, monkeys, rats and cows, but not cell lines from pigs or dogs. Thus, the virus isolated from persistently infected mouse cells had a much broader host range than the parental MHV-A59 wild type virus which can only infect mouse cells. Pretreatment of 17 Cl 1 cells with anti-MHVR MAb-CC1 prevented infection with MHV-A59, but only partially blocked infection with MHV/BHK virus. Soluble MHVR glycoprotein neutralized the infectivity of MHV-A59, but only partially neutralized the MHV/BHK. Thus, MHV/BHK virus differs from the parental MHV-A59 virus in receptor utilization.

We suggest that in persistently infected cultures in which expression of the MHVR receptor glycoprotein is markedly reduced and alternative MHV receptors may also be expressed, virus variants that can utilize alternative receptors have a strong selective advantage. Because hamster cells transfected with MHVR are susceptible to MHV-A59 infection, the virus spike-receptor interaction determines the host range (Dveksler, *et al.*, 1991). The hemagglutinin esterase (HE) protein which is expressed in some coronaviruses, if expressed, can enhance virus binding. We showed that HE is not expressed in MHV/BHK. We sequenced the spike gene of MHV/BHK and three viruses that are recombinants between MHV/BHK and either the parental MHV-A59 virus or MHV from persistently infected murine cells at passage 600. The spike gene of MHV/BHK has 63 point mutations and an insert of 21 nucleotides. A recombinant (Rec2A) that could infect BHK cells retained 24 of these mutations plus the insert in the N-terminal portion of the MHV/BHK spike gene. This suggests that the extended host range of MHV/BHK may be due to these mutations in the S1 domain of the spike where the receptor-binding site is located. These preliminary studies suggest a mechanism by which MHV, a plus-stranded virus that has high rates of mutation and recombination, might evolve to enter a new host. This is an intriguing model for receptor jumping of an emerging virus.